510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE

A. 510(k) Number:
k042448

B. Purpose for Submission:
Clearance of new device

C. Analyte:
Total homocysteine

D. Type of Test:
Quantitative indirect enzymatic assay

E. Applicant:
General Atomics, Diazyme Laboratories

F. Proprietary and Established Names:
Diazyme Enzymatic Homocysteine Assay
Diazyme Homocysteine Controls

G. Regulatory Information:

1. Regulation section:
   21 CFR § 862.1377, urinary homocystine (nonquantitative) test system
   862.1660, Quality control material (assayed and unassayed).

2. Classification:
   Class II and Class I (reserved) respectively.

3. Product Code:
   LPS, urinary homocystine (nonquantitative) test system
   JJX, single (specified) analyte controls (assayed and unassayed)

4. Panel:
   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):
   Diazyme Enzymatic Homocysteine Assay is intended for the *in vitro* quantitative determination of total L-homocysteine in serum and heparin plasma. The reagents can assist in diagnosis and treatment of patients suspected in having hyperhomocysteinemia and homocystinuria.

Diazyme Homocysteine Enzymatic Assay Kit contains a single calibrator. The calibrator is used to generate a calibration point that will be used in the calculation of homocysteine concentrations in unknown serum samples.

Diazyme Homocysteine Enzymatic Assay has controls for normal serum homocysteine level and abnormal serum homocysteine level. The controls are used as reference samples for checking the functionality of the Diazyme Homocysteine Enzymatic Assay.

2. Indication(s) for use:

   Diazyme Enzymatic Homocysteine Assay is intended for the *in vitro* quantitative determination of total L-homocysteine in serum and heparin plasma.

3. Special condition for use statement(s):
   For in vitro diagnostic use only

   For prescription use only in clinical laboratories

4. Special instrument Requirements:
   Cobas Mira clinical chemistry analyzer

I. Device Description

The Diazyme Enzymatic Homocysteine Assay consists of 3 wet reagents and one levels of calibrator. Two levels of control materials are sold separately. The reagents contain S-adenosylmethionine, NADPH, 2-oxoglutarate, homocysteine methyltransferase, glutamate dehydrogenase, adenosine deaminase, s-adenosylhomocysteine hydrolase, and buffers. Information about calibrators and controls is below.

Human source material was tested and found negative for HIV1, HIV2, HBV, and HCV using FDA approved methods.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Catch, Inc. Homogeneous Enzymic Homocysteine Reagent

2. Predicate K number(s):
   k011689

3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Intended Use	For the in vitro quantitative determination of total L-homocysteine in serum and heparin plasma. The reagents can assist in diagnosis and treatment of patients suspected of having hyperhomocysteinemia and homocysteinuria.	Intended to measure total homocysteine quantitatively in human serum and plasma. Homocysteine measurements are used in the diagnosis and treatment of hyperhomocysteinemia.
Type of test	quantitative	quantitative
Specimen matrix	Human serum and heparin plasma	Human serum and plasma
Differences		
Item	**Device**	**Predicate**
Test principle	Indirect measurement of homocysteine by the measurement of the co-substrate conversion product	Indirect measurement of homocysteine by the measurement of the ultimate conversion products of serine/ homocysteine conversion to cystathionine

K. Standard/Guidance Document Referenced (if applicable):

NCCS Guideline EP5-A, Evaluation of Precision Performance of Clinical Chemistry Devices

L. Test Principle:

The Diazyme Enzymatic Homocysteine Assay measures homocysteine concentration indirectly by measuring the co-substrate conversion product by the following set of reactions that are started by the mixture of the 3 reagents and the sample. (Hcy = homocysteine; SAM = S-adenosylmethionine; HMTase = homocysteine methyltransferase, SAH = s-adenosylhomocysteine, SAHase = s-adenosylhomocysteine hydrolase, Ado = adenosine, ADA = adenosine deaminase, GLDH = glutamate dehydrogenase)

$$\text{Hcy (from the sample)} + \text{SAM} \xrightarrow{\text{HMTase}} \text{SAH} + \text{Methionine}$$

$$SAH \xrightarrow{SAHase} Hcy \text{ (feeds back to above)} + Ado$$

$$Ado \xrightarrow{ADA} Inosine + NH_3$$

$$NH_3 + NADPH + 2\text{-Oxoglutarate} \xrightarrow{GLDH} Glutamate + NADP^+ + H_2O$$

The conversion of NADPH to $NADP^+$ is measured spectrophotometrically at 340nm and is indirectly proportional to the concentration of homocysteine in the sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility

Precision was evaluated according to NCCLS guideline EP5-A. To evaluate assay imprecision, one level (7 µmol/L) of control material was assayed in duplicate twice per day for 10 days (total measurements per sample, n = 40), and two levels of control material were measured in duplicate twice per day for 20 days (total measurements per sample, n = 80). The results are summarized below (units = µmol/L):

		Within Run		Total	
Sample	Mean	SD	% CV	SD	% CV
Level 1	7.2	0.16	2.2 %	0.28	4.1 %
Level 2	13.2	0.64	3.0 %	0.72	5.9 %
Level 3	29.1	0.81	1.8 %	0.92	4.0 %

b. Linearity/assay reportable range:

Percent recovery was assessed across the reportable range of the device (7 – 50 µmol/L). A serum sample containing 89 µM homocysteine was diluted with a serum sample containing 7 µM homocysteine to create samples across the measuring range of the device. The samples were measured in triplicate and percent recovery of theoretical was calculated. Results are summarized below (units = µmol/L)

Theoretical [Hcy]	**Observed [Hcy]**	**% Recovery**
7	7.1	101.4 %
12	12.0	100 %
29.5	31.8	107.8 %
46	47	104.3 %
53.4	54.5	102.0 %
71.2	69.7	97.9 %

The observed concentration was plotted against the theoretical concentration and the resulting linear regression statistics were (Observed) = 0.9861x + 1.0073; $R^2 = 0.9975$

c. Traceability (controls, calibrators, or method):

A homocysteine stock solution is prepared by reacting L-homocysteine thiolactone with potassium hydroxide. The concentration of homocysteine in the stock solution is measured spectrophotometrically. One level of calibrator material is prepared gravimetrically from the stock solution by dilution with saline. The targeted value is 29.5 µmol/L homocysteine and is verified by a commercially available assay. The calibrator material is provided with the assay. Two levels of control material are prepared gravimetrically from the stock solution by dilution with saline. The targeted values are 7 and 29 µmol/L homocysteine and are verified by a commercially available assay. The control material is sold separately.

d. Detection limit:

The analytical sensitivity, calculated by adding 3 standard deviations to the mean result of 12 measurements of a commercially available zero calibrator, was calculated to be 0.3 µmol/L homocysteine.

e. Analytical specificity:

The following substances caused <10% interference at the following concentrations when added to a sample containing 12.9 µmol/L homocysteine.

Substance	**Concentration**
NH_4Cl	50 µM
NaP_i	1 mM
NaF	1 mM
Triglycerides	2500 mg/dL
Ascorbic Acid	10 mM
Bilirubin	20 mg/dL
Hemoglobin	1200 mg/dL
Glutathione	0.5 mM*
L-cysteine	1 mM
S-Adenosylmethionine (SAM)	20 µM
Adenosine	100 µM
Cystathionine	20 µM

*Glutathione at 1 mM caused +13.5% interference while glutathione at 0.5 mM causes less than 10 % interference

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

To assess the accuracy of the device, 46 serum samples (19 natural samples, 21 spiked samples, and 6 diluted samples) and 29 plasma samples were measured using the device

and the predicate device. Results were compared and the regression statistics are summarized below:

Serum: (Device) = 1.0293(Predicate) + 1.6231
R^2 = 0.9772
Range tested = 3 – 42 μM

Plasma: (Device) = 0.9752(Predicate) + 0.7382
R^2 = 0.9742
Range tested = 5 – 42 μM

b. Matrix comparison:

Not applicable. The sponsor tested accuracy for both serum and plasma.

3. Clinical studies:
   a. Clinical sensitivity:
   Not applicable

   b. Clinical specificity:
   Not applicable

   c. Other clinical supportive data (when a and b are not applicable):
   Not applicable

4. Clinical cut-off:
   Not applicable

5. Expected values/Reference range:

The sponsor cites the National Health and Nutrition Examination Survey for normal ranges in the US population. The package insert provides the following table (units = μmol/L):

	12 – 19 yrs of age	**≥ 60 yrs of age**	**Cut-off for "high"**
Male	4.3 – 9.9	5.9 – 15.3	≥ 11.4
Female	3.3 – 7.2	4.7 – 11.6	≥ 10.4

The sponsor recommends that users establish normal ranges for the population in their region.

N. Proposed Labeling:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.